# Experimental Transformation of Muscle Fiber Properties in Lobster

Abstract. *Like the chelipeds, the claw closer muscles of the adult lobster are asymmetric (dimorphic). In the crusher claw the closer muscle is composed entirely of slow fibers, and in the cutter claw it has 65 to 75 percent fast fibers and 25 to 35 percent slow fibers. While claw placement in the adult is essentially random, it can be demonstrated in two ways that the muscle fiber properties are not genetically fixed: (i) if one claw is removed in the fourth and early fifth stages, the remaining closer muscle develops all slow muscle fibers, and (ii) if the animals are raised in smooth-bottomed containers, both claws can become cutter types, having closer muscles with more than 50 percent fast fibers. Thus, as in vertebrate skeletal muscle, the properties of lobster closer muscle fibers can be transformed by various experimental manipulations.*

Skeletal muscle fibers have been classified as fast or slow according to their speed of contraction. This dichotomy is by no means rigid; rather, it describes the extremes in a spectrum of fiber types that vary in properties between fast and slow. Moreover, there are a number of "matched" properties that are associated with each fiber type, including contraction speed, tetanic fusion frequency, metabolic pathways employed (*1*), and specific activity of the myofibrillar adenosinetriphosphatase (*2, 3*). Although these characteristics tend to covary according to fiber type, numerous investigations of vertebrate skeletal muscle have demonstrated that many are not rigidly fixed, but can be influenced by a variety of treatments. For instance, many properties of fast muscle fibers will be transformed to those of slow fibers after cross-reinnervation (*4*) or sustained stimulation of the motor nerve (*5*). Likewise, some properties of slow muscle can be transformed into those of fast muscle by cross-reinnervation (*4*), tenotomy (*6*), or interfering with the activity of the muscle (*7*). These changes have been thought to be due to the influence of the treatment on the activity of the muscle (*2*) or to a change in a direct biochemical influence of the motor nerve on the muscle (*8*). Whatever the mechanism, it is clear that the transformation of muscle fibers is due, in part, to a direct influence on genetic expression (transcription) in the muscle fiber nucleus (*9*).

Similar transformations of fiber type have been sought for invertebrate muscle, but it has never been demonstrated that fast or slow muscle from any invertebrate has the plasticity characteristic of vertebrate skeletal muscle in regard to the ability to be changed from one type to the other (*10*). We report on a simple preparation, the lobster claw closer muscle, that has the ability to undergo transformation of muscle fiber properties. The morphological and physiological characteristics of these muscles are not under rigid genetic control during the early postlarval period, and we have demonstrated that it is possible to change the normal course of development of both the fast and the slow muscle fibers.

Lobster (*Homarus americanus* Milne-Edwards) larvae (stages 1 to 3) were reared in kreisels (*11*) to the fourth (first postlarval) stage. At this time the animals resembled diminutive adults (*12*); they were placed in individual trays to monitor growth and to reduce mortality due to internecine behavior (*13*). Animals were fed frozen brine shrimp daily. Four groups were studied: (i) animals receiving no treatment (controls), (ii) animals with right claws removed at stage 4 and again at stage 5, (iii) animals with right claws glued closed at stages 4 and 5, and (iv) animals raised in smooth-bottomed containers. The trays for groups (i) to (iii) contained some crushed oyster shells on the bottom as a substrate; the substrate was eliminated from the trays of group (iv) animals. Claws were removed for analysis from one or more animals of each group during successive growth stages. Closer muscles were immobilized in the open position and fixed with Bouin's solution; 90 muscle fibers were systematically sampled from all areas of the muscle and characterized on the basis of sarcomere length [for methods see (*14, 15*)]. The sarcomere length of lobster muscle fibers is a reliable indicator of the type of fiber; fast fibers have short sarcomeres (2 to 4 $\mu$m) and slow fibers have long ones (> 6 $\mu$m) (*10*).

The claw closer muscles in adult lobsters are asymmetric both morphologically and physiologically. The closer muscle of the crusher claw contains all slow muscle fibers, while the closer muscle of the cutter claw has 65 to 75 percent fast and 25 to 35 percent slow fibers (*14, 16*). Both closer muscles receive a fast and a slow axon (*17, 18*). In adult lobsters these axons each have a unique pattern of regional distribution to the closer muscles (*19*). Moreover, while the closer muscle of the cutter claw is capable of twitching, which results in rapid claw closure (< 20 msec), that of the crusher claw cannot twitch, and claw closure is slow (> 100 msec).

This marked dimorphism of the adult claws into cutter and crusher types is not evident in larval and early postlarval (stages 4 and 5) lobsters. Here the claws are symmetric in external appearance, muscle fiber complement (*15*) (Table 1), and pattern of innervation (*20*). Indeed, during stage 4 and the first 2 to 3 days of stage 5, the two claws are essentially identical in morphology and muscle fiber properties (Table 1). Moreover, their future properties have not been irrevocably determined; if one claw is removed at this time, the remaining claw will develop into a crusher (*21–23*) (Table 2). However, if no claws have been removed before the fourth day of stage 5, claw type becomes fixed and is virtually random, and subsequent removal of a claw will not alter it (*21*). At stage 6, one claw can be identified as the cutter because more than 50 percent of its muscle fibers are fast (have short sarcomeres) (Table 1). The other claw, the putative

Table 1. Fiber composition based on sarcomere length of the paired claw closer muscles in juvenile lobsters.

| Stage | Length of animal (mm) | N* | Percentage of muscle fiber type based on sarcomere length† | | | |
|---|---|---|---|---|---|---|
| | | | Claw 1 (cutter) | | Claw 2 (crusher) | |
| | | | Fast | Slow | Fast | Slow |
| 4 | 12 | 7 | 38 | 62 | 27 | 73 |
| 5 | 14 | 2 | 42 | 58 | 28 | 72 |
| 6 | 17 | 4 | 56 | 44 | 21 | 79 |
| 11 | 32 | 1 | 68 | 32 | 11 | 89 |
| 13 | 39 | 1 | 64 | 36 | 0 | 100 |
| 15 | 55 | 1 | 82 | 18 | 4 | 96 |
| Unknown | 130 | 1 | 63 | 37 | 0 | 100 |
| Unknown | 155 | 1 | 68 | 32 | 0 | 100 |

*N, number of animals; 90 fibers were used from each closer muscle. †Fast fibers had sarcomeres of < 6 $\mu$m; slow fibers had sarcomeres > 6 $\mu$m.



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Table 2. Claw type of juvenile lobsters, based on external morphology, following claw removal or immobilization.

| Animals | $N$* | Right crusher (%) | Left crusher (%) |
|---|---|---|---|
| Control | 21 | 52 | 48 |
| Right claw removed | 23 | 0 | 100 |
| Right claw glued closed | 7 | 43 | 57 |

$N$*, number of animals.

crusher, always has fewer than 35 percent fast fibers (*24*). During the next several molts, the population of fast muscle fibers remains approximately constant in the cutter claw, but steadily declines in the crusher (Table 1).

These observations demonstrate several features of this neuromuscular system. First, both the claw morphology and the closer muscle properties are essentially identical during stage 4 and early stage 5; claw type has not yet been fixed. Second, in stage 6, when claw reversal is no longer possible, the cutter claw has differentiated and is clearly identifiable. Third, the rate of development of the closer muscle is not rigidly controlled; this is particularly true in regard to the crusher claw (Table 1).

The last point was further demonstrated by modifying the conditions under which animals were reared from stage 4 to stage 8. Experimental animals were placed in plastic containers identical to those for control animals but without broken oyster shells. Lobsters typically spent most of their time in a corner of the container; when oyster shells were present, the lobsters manipulated them and often gathered them into this corner. Each of these animals developed both a cutter claw and a crusher claw. When oyster shells were omitted, approximately 30 percent of the animals failed to develop a crusher claw, even as late as stage 15 (approximately 1 year old). From external appearance, these animals had two identical cutter claws (*25*) (Fig. 1). To determine whether the fiber properties of the closer muscles were also identical, we analyzed muscle fibers from three such animals at various stages (Table 3). The results revealed a dramatic increase in the number of short-sarcomere, fast fibers of both claws (Table 3). This increase reflected the transformation of the putative crusher muscle into a cutter-like muscle, which now contained 50 percent fast fibers compared to less than 5 percent in a typical crusher muscle. The putative cutter muscle had about 90 percent short-sarcomere, fast fibers rather than the 70 percent found in a normal cutter muscle (Table 1).

These results demonstrate that claw morphology and the properties of the claw closer muscles are not under rigid genetic control and, indeed, that the normal direction of development can be profoundly influenced. These observations suggest that claw development may depend, at least in part, on claw use and disuse. When a claw is removed, the remaining claw is necessarily used more, and it subsequently develops into a crusher (*26*). Likewise, when animals are raised with no pieces of broken oyster shell to manipulate, neither claw is used extensively and both develop more slowly, often as two cutter claws. However, other results suggest that claw development might well depend on factors in addition to use and disuse. If the right claw is glued closed during stages 4 and 5, there is virtually no influence on claw placement (*27*) (Table 2). It will be of interest to determine the effects of other treatments, such as tenotomy, axotomy, and dactylotomy on claw growth and development.

Previous studies of the development of crustacean fast and slow muscles have suggested that their fiber properties are established at the earliest stages examined, namely before the time of hatching (*28*). Our results demonstrate that the properties of the lobster claw closer muscle are not genetically fixed on hatching and that they can be influenced by various treatments. Thus, we have shown that an invertebrate skeletal muscle has a large degree of plasticity, much like that previously demonstrated for mammalian skeletal muscle. This property has been well known in vertebrate muscle (*2*), but the mechanisms remain obscure. The accumulating evidence suggests that muscle activity is important in this plasticity, although other studies implicate substances released from the presynaptic neuron (*8*) or ion fluxes across the sarcolemma (*29*). Since the closer muscle receives only two motor axons and has only one antagonist, this should be a most interesting system in which to study the interactions between identified neurons and their postsynaptic structures.

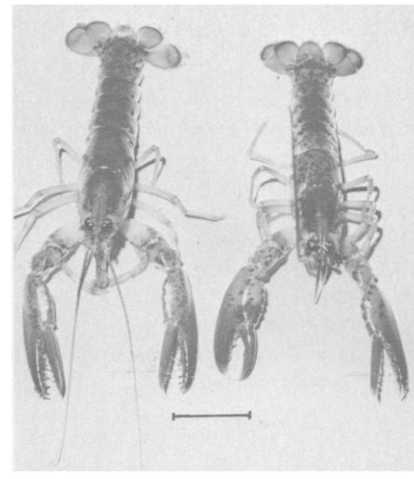

Fig. 1. Two juvenile lobsters, one normal (right) and one with two cutter claws. Both animals were raised in plastic trays under identical conditions, except that the one on the right had some crushed oyster shell in its tray and the one on the left had no substrate in its tray. Both animals are approximately 15 months old and 6 cm in total length (rostrum to telson). Scale bar, 2 cm.

Table 3. Muscle fiber composition, based on sarcomere length, of the paired claw closer muscles in juvenile lobsters raised in smooth-bottomed containers.

| Stage | Length of animal (mm) | $N$* | Percentage of muscle fiber type based on sarcomere length† | | | |
|---|---|---|---|---|---|---|
| | | | Claw 1 (cutter) | | Claw 2 (crusher) | |
| | | | Fast | Slow | Fast | Slow |
| 13 | 35 | 1 | 92 | 8 | 59 | 41 |
| 14 | 45 | 1 | 89 | 11 | 49 | 51 |
| 15 | 55 | 1 | 91 | 9 | 62 | 38 |

*$N$, number of animals; 90 fibers were used from each closer muscle. †Fast fibers had sarcomeres of $< 6$ $\mu$m; slow fibers had sarcomeres $> 6$ $\mu$m.


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19. The pattern of innervation differs for each of the two motor axons in each claw closer muscle. Likewise, the patterns of distribution of the fast and slow axons in the cutter are different from those of their counterparts in the crusher (*18*).
20. Unlike the adult closer muscles (*18*), virtually all muscle fibers in stage 4 claws are innervated by both the fast and slow motor neurons. A few fibers in the proximal region receive only the slow axon (W. J. Costello and F. Lang, unpublished observations).
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23. This type of "reversal" is also observed in a number of other crustaceans. For instance, *Alpheus*, the pistol or snapping shrimp, has a small cheliped and a large snapping cheliped. If the latter is removed, the small claw will transform into a large claw and the regenerating claw will become a small claw. See H. Przibram, *Connecting Laws in Animal Morphology* (Univ. of London Press, London, 1931).
24. We examined four animals in stage 6. Fast (short sarcomere) fibers ranged from 41 to 64 percent (mean, 56 percent) in the cutter and from 6 to 32 percent (mean, 21 percent) in the crusher.
25. This was observed first by J. Hughes of the Massachusetts State Lobster Hatchery, Martha's Vineyard, and later by A. Sastry of the University of Rhode Island School of Oceanography (personal communication). Both have raised such animals to sexual maturity (about 400 to 450 g).
26. The regenerating claw is enclosed in a tough, transparent capsule and cannot be used until after the next molt.
27. This experimental group began with 12 animals. However, there was a higher than normal loss because animals often lost a claw during the molt to stage 5 or 6. Of the seven animals that were successfully reared, four had left crusher claws and three had right crusher claws. While this procedure eliminates shortening of the closer muscle, it probably does not interfere with isometric contraction; the influence on normal motor activity, if any, is not known.
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# Rebound Insomnia: A New Clinical Syndrome

Abstract. *Rebound insomnia followed the withdrawal of three benzodiazepine hypnotic drugs, each of which had been administered in a single nightly dose for only short-term periods. The intense worsening of sleep is attributed to the short duration of the action of these drugs. A hypothesis involving benzodiazepine receptors in the brain is proposed in which there is a delay or lag in replacement of endogenous benzodiazepine-like molecules after the abrupt withdrawal of exogenous drugs.*

We previously reported that a worsening of sleep was associated with the abrupt withdrawal of nonbenzodiazepine hypnotic drugs that had been administered in multiple nightly doses over a long period (*1*). We termed this condition drug-withdrawal insomnia (*1*) and considered it part of a general abstinence syndrome resulting from the withdrawal of depressant drugs and related to supersensitivity in the central nervous system (CNS) (*2*). We now describe a new clinical entity, "rebound insomnia," which consists of a marked worsening of sleep following the abrupt withdrawal of certain benzodiazepine drugs administered in only single doses nightly for short periods.

We have now analyzed data from six separate sleep laboratory evaluations of three benzodiazepine hypnotic drugs (*3–8*); three of these studies, one with each drug, were conducted in our laboratory (*3*, *6*, *7*). According to the data, an intense form of rebound insomnia occurs after the withdrawal of only a single nightly dose of certain benzodiazepine hypnotic drugs that had been administered for short and intermediate as well as long periods.

The study designs all included an initial placebo-baseline period, a short-, intermediate-, or long-term drug administration period, and a placebo-withdrawal period. Each study evaluated only one drug in a fixed dose. All subjects were insomniacs who were continuously monitored by electroencephalogram (EEG), electromyogram (EMG), and electrooculogram (EOG).

Kales *et al.* (*3*) evaluated triazolam (0.5 mg) in seven subjects according to a 22-night protocol including four placebo-baseline nights (the first for adaptation and the next three for baseline measurements), 2 weeks of nightly drug administration, and four placebo-withdrawal nights (Table 1). Although short-

Table 1. Effects of benzodiazepines on the induction and maintenance of sleep. Data are means and standard errors. The total time of recording each night was 8 hours. All statistical comparisons are with the baseline data; the Dunn multiple comparison *t*-test was used for the analyses.

| Condition | Day | Time awake (minutes) | | | Wakings (No.) |
|---|---|---|---|---|---|
| | | Latency to sleep | After sleep onset | Total | |
| | | *Triazolam (0.5 mg) (3)* | | | |
| Baseline | 2 to 4 | 61.4 ± 9.7 | 32.8 ± 6.1 | 94.2 ± 11.8 | 20.4 ± 2.3 |
| Drug administration | | | | | |
| Short-term | 5 to 7 | 35.5 ± 3.2 | 16.2 ± 1.9* | 51.7 ± 3.5† | 14.4 ± 1.0† |
| Intermediate-term | 16 to 18 | 49.0 ± 4.0 | 29.2 ± 4.8 | 78.2 ± 5.8 | 20.3 ± 2.2 |
| Withdrawal‡ | 19 to 21 | 97.3 ± 13.8† | 53.2 ± 9.0* | 150.5 ± 19.3† | 21.5 ± 2.2 |
| | | *Flunitrazepam (1 mg) (6)* | | | |
| Baseline | 2 to 4 | 43.0 ± 4.2 | 33.7 ± 3.4 | 76.7 ± 5.6 | 26.3 ± 1.5 |
| Drug administration | 5 to 11 | 41.3 ± 2.1 | 33.6 ± 3.6 | 74.9 ± 4.5 | 19.9 ± 1.0† |
| Withdrawal | 12 to 14 | 67.7 ± 9.0† | 53.1 ± 10.0* | 122.8 ± 14.2† | 23.6 ± 1.8 |
| | | *Nitrazepam (10 mg) (7)* | | | |
| Baseline | 2 to 4 | 39.6 ± 5.3 | 34.8 ± 3.1 | 74.4 ± 8.7 | 23.9 ± 2.0 |
| Drug administration | 5 to 11 | 26.4 ± 1.5* | 20.1 ± 1.6* | 46.5 ± 2.4† | 17.5 ± 1.2 |
| Withdrawal | 12 to 14 | 38.5 ± 6.2 | 65.3 ± 13.1† | 103.8 ± 15.4† | 22.3 ± 1.9 |

*$P < .05$. †$P < .01$. ‡Only the first three nights of withdrawal were used in this analysis.


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